PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 39/21 // C12N 15/62

A1

(11) International Publication Number: **WO 90/14842**

(43) International Publication Date: 13 December 1990 (13.12.90)

(21) International Application Number: PCT/GB90/00842

(22) International Filing Date: 31 May 1990 (31.05.90)

(30) Priority data:
8912496.0 31 May 1989 (31.05.89) GB

(71) Applicants *(for all designated States except US)*: MEDICAL RESEARCH COUNCIL [GB/GB]; 20 Park Crescent, London W1N 4AL (GB). UNIVERSITY OF READING [GB/GB]; Whiteknights, Reading RG6 2AH (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : ALMOND, Jeffrey, William [GB/GB]; EVANS, David, John [GB/GB]; Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ (GB). McKEATING, Jane, Alison [GB/GB]; WEISS, Robin, Anthony [GB/GB]; Institute of Cancer Research, Chester Beatty Laboratories, Fulham Road, London SW3 6JB (GB).

(74) Agents: WOODS, Geoffrey, Corlett et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5EU (GB).

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB, GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: VACCINE AGAINST HIV

(57) Abstract

A chimaeric protein which presents the CD4 receptor 5 binding site of HIV-1 or HIV-2 is useful as a vaccine against both HIV-1 and HIV-2. Preferably, the CD4 receptor binding site is present at antigenic site 1 of an attenuated type 1 poliovirus.

* See back of page

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MC	Monaco
AU	Australia	FI	Finland	MG	Madagascar
BB	Barbados	FR	France	ML	Mali
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Fasso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GR	Greece	NL	Netherlands
BJ	Benin	HU	Hungary	NO	Norway
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	SD	Sudan
CF	Central African Republic	KP	Democratic People's Republic of Korea	SE	Sweden
CG	Congo			SN	Senegal
CH	Switzerland	KR	Republic of Korea	SU	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
DE	Germany, Federal Republic of	LK	Sri Lanka	TG	Togo
DK	Denmark	LU	Luxembourg	US	United States of America

Vaccine against HIV

The present invention relates to vaccines against human immunodeficiency virus (HIV), more particularly HIV-1 and HIV-2.

HIV preferentially infects cells which carry the CD4 surface antigen. A region of the gp120 glycoprotein of HIV-1 has been shown to be critical for interaction with the CD4 receptor (Lasky et al, Cell 50, 975-985, 1987). This region is referred to here as the CD4 receptor binding site. We have prepared a poliovirus chimaera in which the amino acids of antigenic site 1 of the poliovirus have been replaced by amino acids corresponding to the HIV-1 binding site for the CD4 receptor. Surprisingly, the chimaera was capable of raising antibodies which neutralised both HIV-1 and HIV-2 isolates.

Accordingly, the present invention provides use of a chimaeric protein which presents the CD4 receptor binding site of HIV-1 or HIV-2 in the preparation of a medicament for use as a vaccine against both HIV-1 and HIV-2. The invention also comprises an agent for use as a vaccine against HIV-1 and HIV-2 comprising a chimaeric protein which presents the CD4 receptor binding site of HIV-1 and HIV-2.

The chimaeric protein employed in the present invention is a protein, other than the gp120 glycoprotein of HIV-1 and the corresponding HIV-2 glycoprotein, which has been modified so that its amino acid sequence also comprises the sequence of

the CD4 receptor binding site of HIV-1 or -2. Some of the amino acid residues of a protein may be replaced by those of the CD4 receptor binding site. Alternatively, the amino acid sequence of the CD4 receptor binding site may be fused to a foreign protein. The amino acid sequence of the CD4 receptor binding site is exposed on the surface of the chimaeric protein so that the sequence is presented to the immune system. The carrier protein may take the form of a particle or form part of a particulate aggregation. Such an aggregation may comprise a plurality of chimaeric proteins and/or may be a viral particle. The aggregation may comprise either a single type of chimeric protein or a heterogeneous mixture of two or more types of chimeric proteins.

A protein to which the amino acid sequence of the CD4 receptor binding site may be fused may be a particle-forming protein such as hepatitis B surface antigen (EP-A-0175261). The sequence of the CD4 receptor binding site may be inserted into the sequence of a viral protein exposed on the surface of the virus (GB-A-2125065). The viral protein may be a capsid protein of a virus. The CD4 receptor binding site may therefore be provided at one of the antigenic sites of a picornavirus such as a poliovirus (EP-A-0302801). The CD4 receptor binding site may be presented at one of the antigenic sites, for example site 1, 2 or 3, on a capsid protein of an attenuated strain of type 1 poliovirus, or at an antigenic site of type 2 or 3 polio virus. Other

picornaviruses, suitably modified, may be used, e.g. Bovine enterovirus.

The amino acid sequence of an antigenic site of a picornavirus may be replaced completely or partly by the amino acid sequence of the CD4 receptor binding site. Preferably the CD4 receptor binding site is provided in place of some or all of antigenic site 1 of an attenuated strain of type 1 poliovirus. The attenuated strain is typically the Sabin 1 vaccine strain. Antigenic site 1 of a type 1 poliovirus is composed of amino acid residues 91 to 102 of the VP1 capsid protein.

The CD4 receptor binding site which is presented by a chimaeric protein may be residues 423-439 of gp120 of HIV-1 or the corresponding HIV-2 residues. The numbering of the HIV-1 residues is according to the sequence of the molecular clone NY5/LAV-1 as referenced in "AIDS and Human Retroviruses 1988" compiled by G. Myers, Los Alamos, U.S. The specific amino acids denoted by these residues may vary from isolate to isolate, as shown in Lasky et al, 1987. The residues may be according to the one letter code (Eur. J. Biochem. 138, 9-37, 1984):

HIV-1: NMWQEVGKAMYAPPISG

HIV-2: NTWHKVGRNVYLPPREG

These sequences may be varied by one or more amino acid substitutions, insertions or deletions providing the resulting sequence still acts as a CD4 receptor binding site.

Further, additional amino acids may be provided at either or both ends. Up to 8, for example up to 4, additional amino acids may be provided at the N-terminal end and/or up to 8, for example up to 4, additional amino acids may be provided at the C-terminal end. Typically these are the amino acids naturally flanking residues 423-439 in the gp120 HIV-1 glycoprotein or the corresponding HIV-2 residues. It is also possible to use a hybrid sequence comprising a portion of the HIV-1 sequence mentioned above attached at the N or C terminal end to a portion of the HIV-2 sequence. Selection of appropriate sequences may lead to an improvement of the HIV-1/HIV-2 cross reactivity of antibodies produced in response to such a chimeric protein.

The chimaeric proteins are recombinant proteins. They may be obtained by inserting a DNA fragment encoding the CD4 receptor binding site into a vector at a location which enable the CD4 receptor binding site to be expressed, as part of a chimaeric protein, exposed on the surface of the protein, and expressing the chimaeric protein. Depending on the type of chimaeric protein, the protein may self-assemble into particles.

A cassette vector may be employed into which a DNA fragment encoding the CD4 receptor binding site is inserted such that the chimaeric protein can be expressed. A cassette vector, suitable for use in constructing poliovirus chimaeras, comprises, under the control of a promoter, a full

length infections cDNA of an attenuated strain of type 1 poliovirus having Sal 1 and Dra 1 sites flanking antigenic site 1 of the poliovirus as follows:

92	93			102	103
GTC	GAC	-	X -	TTT	AAA
Sal 1				Dra 1	

where the numbers represent the numbers of amino acids of the VP1 capsid protein and X represents one or more intervening nucleotides of DNA, present in sufficient numbers to allow the vector to be digested with both Sal 1 and Dra 1 the said Sal 1 and Dra 1 sites being the only Sal 1 and Dra 1 sites in the vector.

Using this cassette vector, an amino acid sequence comprising the CD4 receptor binding site can be inserted at antigenic site 1 of the attenuated poliovirus to replace VP1 amino acid residues 94 to 102, thereby obtaining poliovirus chimaeras capable of acting as vaccines. The cassette vector has the additional advantage that the Sal 1 and Dra 1 sites are unique to the entire vector, allowing replacement of the region flanked by these sites in a single step and thus obviating the need for subcloning steps in the construction of recombinant cDNAs. The amino acid change at position 102 from aspartic acid to phenylalanine, resulting from the creation of the Dra 1 site, does not affect viability or growth of the virus.

Preferably the cassette vector comprises an infectious

full length cDNA clone of the Sabin strain of poliovirus type 1 into which the Sal 1 and Dra 1 sites have been engineered. In such circumstances, X represents the codons for amino acid residues 94 to 101 of the VP1 capsid protein of Sabin type 1. It is generally preferred that X represents a DNA sequence encoding VP1 amino acid residues 94 to 101 of the attenuat d strain of type 1 poliovirus being used. X can, however, denote a DNA sequence from which one or more of these codons is missing or, indeed, represent a longer sequence. Typically X consists of from 6 to 30 nucleotides, for example from 9 to 24 nucleotides.

The cassette vector is typically a plasmid. The plasmid generally comprises an origin of replication, so that it is replicates in the host which harbors it. Typically the host is a microbial host such as a strain of bacterium, e.g. E.coli. The plasmid also generally comprises a marker gene such as an antibiotic-resistance gene. A particularly preferred plasmid is pCAS1. E. coli MC1061 harbouring pCAS1 has been deposited at the National Collection of Industrial and Marine Bacteria, Aberdeen, GB on 25th May 1989 under accession number NCIMB 40148.

Cassette vectors according to the present invention are, like pCAS1, generally double-stranded. The nucleotide sequence, and amino acid sequence according to the one letter code, for pCAS1 and other type 1 vectors which do not have missing any site 1 codons in the region of antigenic site 1

is:

```
91                                                  105
 T  V  D  N  S  A  S  T  K  N  K  F  K  L  F
ACCGTCGACAACTCAGCTTCCACCAAGAATAAGTTTAAACTATTT
TGGCAGCTGTTGAGTCGAAGGTGGTTCTTATTCAAATTTGATAAA
    Sal 1                                Dra 1
```

A cassette vector according to the invention may be prepared by first engineering the Sal 1 and Dra 1 sites into a full length infectious cDNA of an attenuated strain of type 1 poliovirus. This may be achieved by subcloning a partial fragment at the cDNA into a single-stranded cloning vector such as one of the M13 vectors and creating the Sal 1 and Dra 1 sites by site-directed mutagenesis using appropriate oligonucleotides. The modified fragment is then reintroduced into the cDNA from which it has been derived.

The cDNA is provided with a suitable promoter, for example a T7 promoter, and is introduced into a vector having no Sal 1 and Dra 1 sites. The vector may be pFBI 2 (Pharmacia) which has been modified to remove its three Dra 1 sites. In order to obtain a cassette vector which does not include the normal codons for VP1 amino acid residues 94 to 101, a cassette vector prepared as just described is digested with Sal 1 and Dra 1 and an appropriate DNA fragment is ligated with the digested vector. Alternatively, such a vector may be obtained by site directed mutagenesis.

Poliovirus chimaeras which present the CD4 receptor binding site at antigenic site 1 are prepared by a process comprising:

(i) constructing a double-stranded DNA fragment which encodes the CD4 receptor binding site and which has a 5'-Sal 1 cohesive end and a 3'-blunt end;

(ii) digesting a cassette vector according to the invention with Sal 1 and Dra 1 and ligating the fragment constructed in step (i) with the digested vector; and

(iii) obtaining live virus from the modified vector obtained in step (ii).

Step (i) is generally conducted by synthesizing complementary oligonucleotides and annealing the oligonucleotides. The oligonucleotides may be boiled together for from 2 to 5 minutes, for example for about 3 minutes, and allowed to cool to room temperature. In step (ii) the annealed oligonucleotides are ligated with a cassette vector which has been digested with Sal 1 and Dra 1 to excise the DNA encoding antigenic site 1. E. coli may then be transformed with the ligation mix and screened for the presence of the recombinant vector.

Live virus is recovered from the modified full length cDNA by production of a positive sense RNA. The vector incorporating the DNA fragment encoding the CD4 receptor binding site is cut by a restriction enzyme outside the cDNA. The promoter controlling transcription of the cDNA then enables RNA to be obtained. A T7 promoter is particularly suitable for directing transcription in vitro (van der Werf et al, Proc. Natl. Acad. Sci. USA 83, 2330-

2334, 1986). The recovered RNA may be applied to tissue cultures by standard techniques (Koch, Curr. Top, Microbiol. Immunol, 61, 89-138, 1973). For example, the RNA can be used to transfect Hep2C monolayers. After 2 to 8 days incubation, from example after 4 to 6 days incubation, virus can be recovered from the supernatant of the tissue culture.

The chimaeric proteins are useful as vaccines against both HIV-1 and HIV-2. An effective amount is administered to a patient requiring vaccination. They may be administered orally, nasally or parenterally for example intravenously, subcutaneously or intramuscularly. The dose of the chimaeric protein depends on a variety of factors including the age and weight of a patient and the type of carrier protein which has been modified so that its amino acid sequence includes the sequence of the CD4 receptor binding site.

Typically, however, from 10 to 1000 μg of protein may be administered for each route of administration. More preferably from 10 to 100 μg may be given. The chimaeric protein may be given once but preferably a second dose is given from 2 to 4 weeks later. In the case of a poliovirus chimaera, a dose corresponding to the amount administered for a conventional live virus vaccine may be given, usually in the range 10^4-$10^8$$TCID_{50}$, and more usually between 10^5 and $10^{6.5}$$TCID_{50}$. The dose will depend in part on the viability and replicative capacity of the virus used.

Pharmaceutical compositions comprising the chimaeric

protein and a pharmaceutically acceptable carrier or diluent are formulated to enable the chimaeric protein to be administered as a vaccine. Any appropriate carrier or diluent may be employed, for example an isotonic saline solution for parenteral administration. A nasal spray may be formulated with a liquid carrier. An adjuvant may be present. A live attenuated poliovirus chimaera may be formulated stabilised in a solution of 1M $MgCl_2$. The type of carrier or diluent will depend upon the nature of the chimaeric protein, but the following ways of formulating vaccines can be adopted as appropriate:

(a) For oral administration, tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs may be formulated. Compositions intended for oral use may be prepared according to any method known in the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations.

Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or

sodium phosphate; granulating and disintegrating agents, for example, maize starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc.

The tablets may be uncoated or they may be coated by kn wn techniques to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glycerol monostearate or glycerol distearate may be employed.

Formulation for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be naturally-occurring phosphatides, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate,

or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as poly-oxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate. The said aqueous suspensions may also contain one or more preservatives, for example ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid. Dispersable powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent and one or more preservatives.

Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above Additional excipients, for example sweetening, flavouring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan mono-oleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, sorbitol and sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents.

(b) For parenteral administration, either subcutaneously, or intravenously, or intramuscularly, or intrasternally, or by infusion techniques, sterile injectable aqueous or oleagenous suspensions may be formulated. Such a suspension may be formulated according to the known art using those

suitable dispersing of wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition fatty acids such as oleic acid find use in the preparation of injectables.

(c) For inhalation, aerosols or solutions for nebulizers may be formulated.

The following Examples illustrate the invention. A Reference Example is provided.

Reference Example: Construction of cassette vector pCAS1

Taking advantage of codon degeneracy, the nucleotide sequence of Sabin 1 cDNA in the region 2740-2800 was searched for sequences at which restriction endonuclease sites unique to the cDNA could be introduced with minimal alteration to the amino acid sequence. It was observed that a Sal 1 site at nucleotide 2753 could be created without alteration to the amino acid sequence and that this site would be unique within the virus sequence. Similarly a unique Dra 1 site could be

created at position 2783 resulting in the replacement of aspartic acid (VP1 residue 102) by phenylalanine.

The synthetic oligonucleotides 5'-GGAAGCTGAGTTGTCGACGGTTATAATGG-3' and 5'-CACTGTAAATAGTTTAAACTTATTCTGG-3' (bases inducing changes underlined) were used to create Sal 1 and Dra 1 restriction sites at positions 2753 and 2783 respectively on a 3.6kb Kpn 1 partial fragment (nucleotides 66-3660) of an infectious Sabin 1 cDNA (Stanway et al, J. Virol. 57, 1189-1190, 1986) subcloned in M13mp18, using the gapped-duplex mutagenesis technique (Kramer et al, Nuc. Acids Res. 12, 9441-9456, 1984). The alterations made to the antigenic site were confirmed by dideoxy chain termination sequencing.

The nucleotide and amino acid sequence of poliovirus Sabin 1 illustrating changes introduced in the construction of pCAS1 are shown below. Nucleotides 2750-2794 of the cDNA sequence of the viral sense strand are shown, together with the location of the introduced restriction sites. The resulting amino acid change to phenylalanine from aspartic acid at position 102 is shown in parenthesis.

ANTIGENIC SITE 1

```
91                                                  (F)          105
:                                                                :
T   V   D   N   S   A   S   T   K   N   K   D   K   L          F
ACC GTG GAT AAC TCA GCT TCC ACC AAG AAC AAG GAT AAG CTA TTT
    GTC GAC                                 TTT AAA
    Sal 1                                   Dra 1
```

The mutated fragment was introduced into a full-length 1 cDNA of Sabin type 1 onto which a T7 promoter had previously been engineered at the extreme 5' end. This full-length clone was subsequently transferred into vector pFBI 2 (Pharmacia), which had been modified to remove its 3 Dra 1 sites at positions 2052, 2071 and 2763, by insertion of an Eco R1 linker following Dra 1 digestion. An Eco R1 - Sal 1 fragment carrying this modified full-length poliovirus clone was ligated into Eco R1-Xho 1 digested pFBI 2-derived vector thereby destroying this Sal 1 site. The resulting plasmid, pCAS1, therefore contained a full-length Sabin 1 cDNA under the control of a T7 promoter and in which the introduced Sal 1 and Dra 1 sites were unique.

Recovery of infectious virus from Nael linearised pCAS1 was achieved following transfection of Hep 2C monolayers with transcripts produced in vitro by T7 RNA polymerase (Stratagene) as previously described (van der Werf et al, Proc. Natl. Acad. Sci. USA 83, 2330-2334, 1986). The genomic sequence of recovered virus was verified by primer extension sequencing of viral RNA (Rico-Hesse et al, Virology 160, 311-322, 1987). The single substitution of aspartic acid for phenylalanine at residue 102 had no apparent affect on virus viability. Furthermore the design of the cassette was such that the altered amino acid would be lost upon insertion of replacement sequences.

Example 1: Construction of a chimaeric poliovirus containing residues 423-439 of the glycoprotein gp120 of HIV-1

100 ng each of complementary oligonucleotides encoding the HIV-1 sequence of choice were boiled for three minutes and allowed to cool to room temperature. The oligonucleotides were:

TCGACAACATGTGGCAAGAGGTAGGTAAGGCAATGTACGCTCCACCAATTTCAGGT

GTTGTACACCGTTCTCCATCCATTCCGTTACATGCGAGGTGGTTAAAGTCCA

Aliquots of this annealed mix were then ligated with Sal 1 - Dra 1 digested pCAS1. Competent E. coli were transformed with the ligation mix and recombinant plasmids screened for the presence of the HIV sequence inserted. The resulting recombinant plasmid, pSI/env/4 was linearised with Nae 1, which cuts within vector sequences of the construct, and used as a template in a T7 transcription reaction (van der Werf et al, Proc. Natl. Acad. Ser. USA 80, 5080-5084, 1983) prior to transfection of sub-confluent Hep2C monolayers.

After three to four days a cytopathic effect was observed. The RNA sequence of approximately 200 bp spanning antigenic site 1 of the recovered chimaeric virus SI/env/4 was confirmed by primer directed chain termination sequencing. The nucleotide and amino acid sequence of the region of antigenic site 1 of pCAS1 and of the corresponding region of pSI/env/4 are shown below.

91 105

pCAS1 T V D N S A S T K N K F K L F

ACCGTCGACAACTCAGCTTCCACCAAGAATAAGTTTAAACTATTT

Sal 1 Dra 1

S1/env/4

T V D N M W Q E V G K A M Y A P P I S G K L F

ACCGTCGACAACATGTGGCAAGAGGTAGGTAAGGCAATGTACGCTCCACCAATTTCAGGTAAACTATTT

TGGCAGCTGTTGTACACCTGGCTCCATCCATTCCGTTACATGCGAGGTGGTTAAAGTCCATTTGATAAA

Example 2: Neutralization of HIV-1 and HIV-2 isolates

Rabbit antisera were raised to poliovirus chimaera S1/env/4 by inoculation of approximately 0.5 ml of tissue culture supernatant from Example 1 in adjuvant, and boosted two to four times in a similar manner. Antisera were also raised by injecting rabbits with about $10^8 TCID_{50}$ of purified S1/env/4.

Neutralization titres were determined by incubation 10 μl of heat inactivated antiserum with 40 μl of virus supernatant containing 10^3 infectious units of HIV at 37°C for 1 hour. The results are shown in Table 1 below and are expressed as the reciprocal of the serum dilution giving > 90 % reducti n in HIV infectivity. Antisera R10, R11 and R12 are sera raised using tissue culture supernatant for immunizations. Antisera R19 and R20 are sera raised using purified S1/env/4 as immunogen nt = not tested.

Table 1: Reciprocal Neutralization Titre

Anti-serum	Virus strain											
	HIV-1							HIV-2				
	IIIB	RF	SF2	SF33	CBL4	Z84	Z129	LAV2	CBL20	CBL21	CBL22	CBL23
R10	20	40	nt	nt	20	20	10	10	nt	20	<10	nt
R11	40	40	nt	nt	10	40	40	10	nt	<10	<10	nt
R12	20	10	nt	nt	<10	<10	<10	<10	nt	<10	<10	nt
R19	40	20	80	80	<10	nt	<10	20	<10	20	<10	<10
R20	40	10	40	40	<10	nt	<10	20	<10	<10	<10	<10

CLAIMS

1. Use of a chimaeric protein which presents the CD4 receptor binding site of HIV-1 or HIV-2 in the preparation of a medicament for use as a vaccine against both HIV-1 and HIV-2.

2. Use according to claim 1, wherein the chimaeric protein takes the form of a particle or forms part of a particulate aggregation.

3. Use according to claim 2, wherein the aggregation is a viral particle.

4. Use according to claim 3, wherein the sequence of the CD4 receptor binding site is present in the sequence of a capsid protein of an attenuated virus.

5. Use according to claim 4, wherein the CD4 receptor binding site is presented at one of the antigenic sites on a capsid protein of an attenuated strain of type 1 poliovirus.

6. Use according to claim 5, wherein the CD4 receptor binding site is provided in place of some or all of antigenic site 1.

7. Use according to claim 6, wherein the CD4 receptor binding site replaces amino acid residues 94 to 102 of the VP1 capsid protein of an attenuated strain of type 1 poliovirus.

8. Use according to claim 1, wherein the CD4

receptor binding site is the HIV-1 site having the sequence NMWQEVGKAMYAPPISG or the HIV-2 site having the sequence NTWHKVGRNVYLPPREG.

9. An agent for use as a vaccine against HIV-1 and HIV-2 comprising a chimaeric protein which presents the CD4 receptor binding site of HIV-1 or HIV-2.

10. A method of vaccinating a patient against both HIV-1 and HIV-2, which method comprises administering thereto an effective amount of a chimaeric protein which presents the CD4 receptor binding site of HIV-1 or HIV-2.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/00842

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 39/21, // C 12 N 15/62

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System [8]	Classification Symbols
IPC[5]	A 61 K,C 12 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [9]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A, 0302801 (INSTITUTE PASTEUR) 8 February 1989 see page 2, line 1 - page 4, line 16; page 5, lines 35-44; page 14, lines 6-55 cited in the application	1-9
Y	--	8
Y	Cell, volume 50, 11 September 1987, Cell Press, L.A. Lasky et al.: "Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor", pages 975-985 see the whole article, esp. page 982; figure 9 cited in the application	8
X	-- WO, A, 8707616 (BIOGEN N.V.) 17 December 1987 see page 11, lines 25-31; page 12, lines 1-31; page 21, claims 1-4; page 23, figure 1/1	1,2,8,9
	-- ./.	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18th September 1990	11. 10. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Mme N. KUIPER

International Application No PCT/GB 90/00842

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X,P	EP, A, 0328403 (UNITED BIOMEDICAL INC.) 16 August 1989 see page 6, lines 44-58; pages 12-13, example 5; pages 14-15, claims --	1,2,8,9
A,P	Nature, volume 339, 1 June 1989, D.J. Evans et al.: "An engineered poliovirus chimaera elicits broadly reactive HIV-1 neutralizing antibodies", pages 385-388 see the whole article --	1-9
A	EP, A, 0279688 (GENENTECH INC.) 24 August 1988 see the whole document --	1-9
A	Nature, volume 332, 3 March 1988, K.L. Burke et al.: "Antigen chimaeras of poliovirus as potential new vaccines", pages 81-82 see the whole article --	1-9
A	EP, A, 0243029 (UNITED STATES OF AMERICA) 28 October 1987 see the whole document	

FURTHER INF RMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 10, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods

2. [ ] Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9000842 SA 37394

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 08/10/90 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0302801	08-02-89	FR-A,B	2619012	10-02-89
		AU-A-	2269188	09-03-89
		WO-A-	8901516	23-02-89
		JP-A-	1157380	20-06-89
		OA-A-	8749	31-03-89
WO-A- 8707616	17-12-87	AU-A-	7540487	11-01-88
		EP-A-	0269712	08-06-88
		JP-T-	1501547	01-06-89
		US-A-	4943627	24-07-90
EP-A- 0328403	16-08-89	JP-A-	2022296	25-01-90
EP-A- 0279688	24-08-88	JP-A-	1125327	17-05-89
EP-A- 0243029	28-10-87	AU-A-	7284287	09-11-87
		JP-T-	63501125	28-04-88
		WO-A-	8706262	22-10-87